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A SURFACE STERILISATION TECHNIQUE USED IN FEEDING ALGAE TO ORIBATEI

By

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INTRODUCTION

Although many investigators have cultured soil Oribatid mites, only Woodring and Cook (1962) and Hartenstein (1962) have succeeded in obtaining surface sterile specimens: the former by washing eggs in 1% sodium hypochlorite; the latter by immersion of adult specimens in 70% alcohol after 7 or 10 days of starvation. The following paper describes a technique of surface sterilisation which has proved successful in the case of the Primitive Oribatids and the Phthiracaridae.

METHODS

Mites were extracted in Tullgren funnels and then starved until no more food boli were seen in the gut under ×20 magnification, or for ten days where the integument was too opaque for internal observation. After much experimentation surface sterilisation was finally done by washing the mites in a 1% solution of sodium hypochlorite followed by distilled water. During this process the mites were kept in funnels lined with filter paper held in the necks of 500 ml flasks. Washing was repeated on each of three consecutive days. All work was carried out under sterile conditions. It was also difficult to find suitable culture chambers. Small round Pyrex glass containers with a tightly fitting circle of glass fibre filter paper at the base (Hayes 1963) were eventually used. These were covered by taffeta or other non-hygroscopic fabric permeable to air and secured by a rubber band. Soil algae were provided as food. These were obtained from the Culture Collection of Algae and Protozoa, University of Cambridge. The pots were then placed in desiccators which had been sterilised by standing full of 70% alcohol for 24 hours. The desiccators were lined with blotting paper cut so that wicks hung down into sterile distilled water below. This provided an atmosphere of 100% R.H. near the shelf. The cultures were kept in the light at room temperature.

THE ALGAE AS A FOOD SOURCE FOR SOIL ORIBATEI

Lund (1967) in his review "Soil Algae" states that algae are found in soils everywhere and that although satisfactory methods for estimating productivity have not been devised. Russian work suggests that the amount of organic matter produced by algae can be very considerable and that their importance in soil processes has been underestimated.

However, there is little data available on the numbers of algae in forest soils although many figures are available for other soils. Petersen (1935) found 10 000 algal cells per gram of soil at the surface in a forest at Ermelunden in Denmark. The substrate was loose mould above clay mixed with sand pH 5. At 5 cms depth no algal cells were found but at 10 cms 300 cells were found per gram of soil. Féher (1933) found 793 000 algal cells per gram of soil in a forest in Hungary. He does not state at which depth. Further quantitative data on soil algae of conifer forests in the New World is given by Olsen (1961). Lund (loc. cit.) states that algae are most abundant at or close to the surface but may also be found

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in the lower horizons of the soil. Sometimes the largest population is found a few cms below the surface (BRISTOL ROACH 1927).

In the same review Lund points out that the interrelations between algae and other organisms are little known and that grazing has scarcely been studied. SCHUSTER (1956) found that the gut contents of some *Belba* species contained algae.

Several authors have found green algae a satisfactory food for culturing soil Oribatid mites. PAULY (1956) cultured *Belba geniculosa* (Oudms.), *Belba clavipes* (Herm.), and *Belba gracilipes* (Kulcz) on algae taken from the washings of tree bark. SENGBUSCH (1954) cultured immature stages of *Galumna nervosus* (Berlese), *Galumna longiplumus* (Berlese) and *Galumna elimatus ithacensis* (Jacot) on "Protococcus", a green alga.

Soil mites have been reared on lichens. ROCKETT and WOODRING (1966) cultured *Ceratozetes jeweli* (n. sp.) on chopped up lichen and WOODRING and COOK (1962) also used lichen in the rearing of *Oppia neerlandica* (Oudms.), *Ceratozetes cisalpinus* (Berlese), and *Scheloribates laevigatus* (Koch). GRANDJEAN (1950) observed that *Camisia segnis* (Herm.) would feed on a grey lichen in its natural state.

The species shown in table 1 produced eggs and larvae when fed on a mixture of green algae scraped from tree trunks.

TABLE 1. Species and stages of oribatid mites feeding in culture on algal scrapings from bark

Species	Adult	Egg Production	Larval Production	Larval Feeding
Camisia segnis (Herm.)	×	×	×	×
Camisia spinifer (Koch)	×	×	×	×
Platynothrus peltifer (Koch)	×	×	×	×
Ceratoppia bipilis (Herm.)	×	×	×	—
Porobelba spinosa (Sell.)	×	—	—	—
Adoristes ovatus-poppei	×	—	—	—
Damaeus onustus Koch	×	×	×	×
Odontocepheus elongatus (Mich.)	×	—	—	—

An indication that soil algae may be a source of food for these mites is that species are often found clustered round a group of green algal cells in collecting dishes at the base of Tullgren funnels. However, this is only offered as an indication since *Ceratoppia bipilis* (Herm.) has been seen feeding on the body contents of dead Collembola in collecting dishes and there is no evidence in the literature that this mite is necrophagous.

However, neither the evidence from the literature nor from my early cultures does more than suggest that the algae might form a food source for, as far as can be ascertained, no soil mites have yet been reared successfully under aseptic conditions. WOODRING and COOK (1962) attempted to rear *Ceratozetes cisalpinus* aseptically but were unsuccessful. Thus any food which appears to be palatable to mites may only be a substrate for bacteria or fungi which may be the true food source.

Thirty individuals of *Camisia segnis*, obtained from a stand of Scots Pine interspersed with Larch growing on the slopes of the Pentland Hills south of Edinburgh, were surface sterilised as described earlier and placed in six sterile pots, five individuals to a pot. Four pots were provided with soil algae stated to be free of bacteria and fungi and obtained from the Culture Collection of Algae and Protozoa. The animals in the last two pots were not supplied with food.

For approximately three weeks after treatment with sodium hypochlorite the ani-

mals did not feed. It was thought therefore that the animals' digestive systems might have been damaged by the treatment and, in order to test this, some algal scrapings from tree bark, known to be very acceptable to this species under non-sterile conditions, were provided. Feeding commenced almost immediately on the new substrate and possibly on the soil algae followed by egg and larval production.

The experiment was repeated using only one animal per pot. The cultures remained sterile for $4^1/_2$ weeks. Scrapings from these culture pots were tested on malt agar at the end of this period and found to be free of contamination. The animals did not feed throughout the whole of this period (the criterion of feeding being the production of faecal pellets). When a few untreated mites of the same species were placed in a culture pot with the soil algae mentioned above feeding commenced after four days.

DISCUSSION

The success or otherwise of using liquid as a sterilant for mites may be connected with the extent of the tracheal apertures (MADGE 1964) for those species which can be successfully sterilised in this way are in most cases known to have a reduced tracheal system and also a highly sclerotised integument.

It appears from these preliminary investigations that either mites or food must be contaminated with microorganisms before feeding will take place. When surface sterile mites were provided with food which was free of bacteria and fungi no feeding occurred. It is probable that the techniques of surface sterilisation did not affect the gut symbionts in the first investigation as the animals later fed and reproduced normally. Therefore it appears that contamination of the food is likely to be the important factor. If this is so, it is possible that the food is not recognised as such unless microorganisms are present on it. Alternatively perhaps the microorganisms might break down the food in some way and so render it useable to the animal. It is difficult to conceive that contaminating microorganisms might act upon cells which appear to be living and healthy; but perhaps the microorganisms might break down the food after ingestion and comminution by the mite. WOODRING and COOK (1962) found that a fungal meal was necessary before the larva of *Ceratozetes cisalpinus* would feed or develop.

If it is the mite itself which must be contaminated before feeding can occur then any surface contaminants would presumably fulfil the same function as contaminants of the food. An equally likely suggestion is that a symbiotic microflora is the pre-requisite. It is unknown exactly what effect the surface sterilisation technique has on the gut symbionts if present. BROOKS (1963) states that Rickettsiae have been found acting as symbiotic microorganisms in some species of Acarina. HARTENSTEIN (1962) was unable to demonstrate conclusively the presence of a symbiotic gut flora in *Proctoribates lophotrichus* (Berlese).

SUMMARY

A simple method of surface sterilisation of some species of mites is described. Some evidence is put forward in favour of algae as a food source together with an account of early preliminary investigations which suggest that algae may only be palatable in the presence of microorganisms.

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